NEMATOCYSTS OF MICROSTOMA.[1]

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For the past three autumns many brown *Hydras* and *Microstomas* were found in a fish pond northeast of the University of Virginia. In both these forms of animals were present oval, refractive bodies, which were the nematocysts. These nematocysts when everted have in both cases slender filaments with three barbules radiating from their base. The filament at its base is attached to the neck of a pear-shaped "poison-sack" or capsule. Since Oersted (1844) nematocysts were known to occur in flatworms as well as in Cœlenterata. Associated with the nematocyst of *Hydra* there is a cell which has elaborated the nematocyst and cares for it until it is discharged. This cell is known as a *cnidoblast* or *nematocyte*. Each cnidoblast of *Hydra* has a small spine-like structure—the cnidocil. This is supposed to receive the stimulus that results in the discharge of the nematocyst. When one examines the living *Hydra* and the living *Microstoma* found in this vicinity no difference can be detected between the nematocysts of the two forms, except that there is no cnidocil associated with the nematocysts of the flatworm. Moreover the nematocysts in both *Hydra* and *Microstoma* when undischarged and when being discharged appear to be normal parts of the respective animals. This indigenous appearance and ready discharge of the nematocysts of *Microstoma* led men to look upon them as structures elaborated by the cells of *Microstoma*. Such was my view after I had repeatedly, during three years, studied the nematocysts of living *Microstoma*. According to Martin (1908) until 1903 all zoölogists held that the nematocysts of *Microstoma* were its own products.

Recently the inference has been made that the nematocysts of *Microstoma* have been derived from ingested cœlenterata, as Grosvenor (1903) had claimed for the nematocysts of æolids. Martin (1908) must be given the credit for having first approached this question of the origin of the nematocysts of *Microstoma* in the proper way, *i. e.*, experimentally.

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Martin first made the observation that *Microstoma* do ingest *Hydras*. "If a fasting *Microstoma* is placed in a watch-glass which contains some small *Hydra*, it is almost certain in a short time to come in contact with one of them. If the *Microstoma* comes suddenly against the tentacles of the *Hydra* it contracts itself immediately, and in this condition it may frequently be killed by the discharged nematocysts. As a rule, however, the *Microstoma* fixes itself for a short time by its posterior end in the neighborhood of the *Hydra*, and everts its pharynx to its full extent.

"The *Microstoma* then swims over the surface of the *Hydra*, usually attacking the lower part of its body with its pharynx fully everted (vide Fig. 10). The *Hydra* then usually becomes strongly contracted, and sweeps its tentacles over to the side on which it has been attacked, though under these conditions the tentacles do not grasp the *Microstoma*, but remain extended almost parallel with its body, and it would appear as though the pharyngeal secretion had a paralyzing action on the *Hydra*. In many cases, after a time, the *Microstoma* leaves its prey, and in such a case the *Hydra* does not seem much the worse for the attack, but if the *Hydra* is of small size, it may be engulfed and swallowed whole" (Martin, 1908, pp. 267–8).

After this observation Martin's chief evidence is based upon the histology of *Microstoma*. He finds that when both *Hydra* and *Cordylophora* are fed to *Microstoma* there are found within the tissues of the flatworm nematocysts peculiar to both kinds of cœlenterata that had been fed. Thus in sections of the *Rhabdocæle* he discovers nematocysts in the endoderm, mesoderm, and ectoderm. Martin's methods of procedure had convinced me that he had established that the nematocysts of *Microstoma* were derived from *Hydra*, until I had considered the details of his work.

I hesitate, for example, to follow Martin when he says that von Graff (1882) was wrong in saying that each nematocyst lies in a single cell and that "Hallez had correctly described the nematocysts as lying in vacuoles" (Martin, 1908, p. 263). It had been the experience of students in my classes to find discharged nematocysts *enclosed within a single cell* (Fig. 1). Such isolated nematocysts were obtained by adding a drop of 1 per cent. acetic acid to a slide containing specimens of living flatworms. Some time after a cell of this kind had lain in the dilute acetic acid a large vesicle appears at the pole from which the discharged nematocyst must emerge (Fig. 2). Such distortion has frequently been observed under similar conditions.

So much for my observations upon the conduct and anatomy of living specimens and upon temporary preparations.

Before taking up the evidence afforded by permanent histological preparations I must interpolate my hesitancy, at least, to follow Martin when he says, "I have found very young *Microstoma* in which nematocysts were already present, and at first this presented a difficulty to this theory of the derivation of nematocysts, but I do not believe this is insuperable when we consider that nematocysts in the case of an animal which has fed largely on *Hydra* can be found in almost any tissue of he body. I have found them in the testes (Fig. 8), and although I have not yet found them in an ovum, I believe that the yolk-cells might readily carry them into the cocoon thus causing the infection of the young forms" (Martin, 1908, p. 271). It does not appeal to my judgment that nematocysts with cnidoblasts should be able to "infect" the young forms through the yolk-cells. Nematocysts *within* vacuoles, without attending cells to propogate them, *infecting* young forms through yolk-cells seem to be altogether improbable.

When I consider this last statement of Martin and note his oversight of the cells that in *Microstoma* do enclose some of the nematocysts I am led to believe that the final word has not been spoken concerning the nematocysts of *Microstoma*.

Such was my attitude when during September, October, November and early December, 1910, collections of *Microstoma* were made with a view to studying their histology.

Many specimens were fixed in chrom-aceto-formal; others were fixed in saturated solution of corrosive sublimate to which 5 per cent. of glacial acetic acid had been added. Both of these fixing fluids gave good results. The sections were mounted serially. Some individuals were cut 3 microns, others at 4, 5, 6 and 7 microns. All the material was stained with Haidenhain's iron hæmatoxylin. Some sections were counter stained with Bordeaux red. From these many individuals a large list of sectioned nematocysts has been made.

As the specimens were being collected it was not an unusual experience to find greatly gorged specimens, which upon the addition of the fixing fluid would burst and thus liberate or expose a dead *Chætogaster* such as is represented in Fig. 3. In passing, this observation is of interest, for Martin says "one of the commonest enemies of *Microstoma* appears to be *Chætogaster*, which devours it greedily" (Martin, 1908, p. 268).

Within the enteron of the sectioned *Microstoma*, one frequently finds the large unbroken setæ of these ingested oligochætes (Fig. 4). If according to Martin, as I interpret him, the passage of the nematocysts from the enteron through endoderm and mesoderm to ectoderm is passive, I wonder why at times these harsh setæ of *Chætogaster* do not passively find their way into the tissues of the body of the flatworm. Other solid bodies such as diatom shells, and round or spheroidal objects such as the shells of *Arcella* lie within the enteron. These too, if the migration of the nematocysts be passive, should find their way to the surface as do the nematocysts; but not a single instance of such objects lying outside the lumen of the enteron was observed.

If, therefore, nematocysts do migrate from the cavity of the enteron to the general surface of the body, their migration seems to be accomplished through some active agency.

The first tissue of course to be concerned in this selective function is the endoderm. The cells of the endoderm must distinguish between the nematocysts and other solid or rounded objects within the enteron, just as an *Amœba* distinguishes between food and non-food. So far as the present series of observations is concerned twenty endodermal cells were found in which a nematocyst was enclosed (Fig. 5). In each case the nematocyst lies within a vacuole near the base of the endodermal cell.

The question at once arises, might not this nematocyst have wandered from the mesoderm into the endoderm? There is ample evidence of nematocysts migrating from one region to another in the bodies of cœlenterata. Schneider says: "Alle Nesselzellen der Siphonophoren enstehen an localisierten Bildungsherden, von denen sie in einen bestimmen Entwickelungstadium als Wanderzellen auf die Verbrauchstätten überwandern" (Boulenger, 1910, p. 764). In this connection "Hadzi's results are of the greatest interest, as he was able to examine living tissue as well as preserved material. His main conclusions are as follows:

"1. The thread-cells of hydroids are not formed 'in situ' but in the ectoderm of the cœnosarcal branches, where, on account of the thick perisarcal investment, they can obviously not become functional.

"2. When completely developed, except for accessory structures such as the cnidocils and the stalks, they migrate to the important nematocyst batteries on the tentacles. This migration can take place in two different manners. In simple forms, *e. g.*, *Campanularia*, the thread cells move actively by means of their pseudopodia, making their way between the ectodermal cells of the colony. In *Tubularia*, however, they adopt a quite different method of locomotion: from the ectoderm of the cœnosarc they force a way through structureless lamella and endoderm into the cavity of the hollow stem, whence they are carried by the current caused by the flagella of the endoderm cells to the hydranths. Here the thread-cells re-enter the tissues and migrate actively by their own movements to the ectoderm of the tentacles" (Boulenger, 1910, p. 764). Conklin (1908) likewise observed the formation of nematocysts within the mesoderm of Actinia and their subsequent migration to the ectoderm. Boulenger states "that in *Mœrisia* the nematocysts of the oral battery of the medusa are developed in the endoderm at the base of the manubrium; this does not necessarily imply that the nematoblasts are themselves of endodermal origin" (Boulenger, 1910, p. 767). Thus we have much testimony as to the migration of nematocysts within the tissues of cœlenterata.

It is to be noted, however, that *in all these cited examples the* *nematocysts are transported while enclosed by cnidoblasts.* In the twenty nematocysts found within the endoderm my material shows the stinging-threads lying free within vacuoles at the bases of the endodermal cells. Martin also describes the nematocysts that he noted within the endoderm of *Microstoma* as being free from nematocytes or cnidoblasts. In these cases, therefore, either the cnidoblasts after having carried the nematocysts into the endoderm have died or the stinging-cells were not taken into the endodermal tissue by cnidoblasts. Judging from what has been seen I am of the opinion that these nematocysts have found their way into the endodermal cells through the selective action of the latter. Such selective faculties have also been observed for the cnidophages of the ceras of æolids: Foreign bodies within the lumen of the cnidophore of æolids have been found "by Heckt, by Hancock and Embleton, and by Grosvenor. I have found uninterpretable fragments of tissue, bodies not tissue-like, and diatoms, in the liver diverticula and in the cnidophores. The ciliated canal has no power to distinguish other indigestible bodies from nematocysts, but this inability to select is not shared by the cnidophages, for so far as I know, these ingest only nematocysts" (Boulenger, 1910, pp. 127–8).

The cnidophages of æolids deliver their nematocysts to the cnidocyst, whereas the endodermal cells of *Microstoma* deliver their nematocysts to the mesoderm.

In the material of this laboratory numerous nematocysts are found within the mesoderm. The nematocysts in the mesoderm that lie nearest the endoderm are in all cases enclosed in a vacuole. These vacuoles are similar to those described and figured by Martin and Vallez. About each vacuole the mesoderm crowds. Two kinds of cells of the mesoderm are to be found in or near the walls of these vacuoles: (*a*) a branched type of cells, which form the frame-work of the mesoderm. These cells have nuclei whose chromatin granules are conspicuous but more or less equal in size. The cytoplasm of this first type of cell is not very dense and is greatly branched, its branches anastomosing with those of its fellows (Fig. 6, A); (*b*) cells which are much less frequent in their occurrence, with denser, more compact cytoplasm than that of the first type of cell, the nuclei of the second kind of cells being characterized by having a relatively large black nucleolus which lies in a vacuole (Fig. 6, *B*). These are taken to be the wandering cells or amoebocytes of *Microstoma*.

When the nematocysts are found lying nearer the ectoderm cytoplasmic strands appear extending more or less into the vacuoles about the nematocysts. At *P*, in Fig. 6, is represented what appears to be pseudopodia of a wandering cell invading a vacuole.

Whether or not in this and similar vacuoles there is evidence of amoebocytes entering the vacuoles, the *vacuoles* in all cases are filled by a cell when the nematocysts come to lie near the ectoderm. About the nematocysts that have but recently reached the ectoderm the cytoplasm and nucleus of the invading cell are much more evident than in the cases where the nematocysts have been at the surface sufficiently long or near to suffer discharge (Figs. 7 and 8). In other words the cells seem to spend their vitality in caring for these exotic nematocysts and about the partially discharged nematocysts are to be found cytoplasm and nuclei which indicate a depleted condition (Figs. 9 and 10).

The nuclei of those cells do not lie at the surface of the vacuole as Martin shows in his figure number four, plate 14, as he describes in his legend for this figure. The nucleus in each case can be seen to lie well within the lumen of the original vacuole. This may be determined by the study of transverse or longitudinal sections. Figs. 7 and 8 represent two contiguous sections of a nematocyst near the ectoderm. Fig. 7 was taken through or near the equator of the nematocyst. Fig. 8 represents the section taken immediately following that shown in Fig. 7. In the eighth figure the lower surface of the section is shown. When the focal plane is raised above this level three microns the nuclei of the overarching mesodermal cells appear and the nucleus of the invading cell disappears. Thus it is determined beyond doubt that there is intimately associated with the exotic nematocysts, when they come to lie near the ectoderm, a cell which has invaded the vacuole, that had appeared about the nematocyst within the endoderm. This cell can be seen in fresh material as described earlier in this paper and in all of my permanent preparations.

The question naturally presents itself as to what kind of cell it is that has thus entered the vacuole. In all favorable nuclei that are found within the vacuole there is present a deeply staining nucleolus about which there is to be seen a vacuole (Figs. 8 and 10). This makes the nuclei more closely resemble those of the amœbocytes than those of the other mesodermal cells of the *Microstoma*. So I conclude that in each case an amœbocyte enters the vacuole to take charge of the nematocyst as do the cnidophages of the æolids.

It is interesting to observe how these nematocysts are handled by the invading cell of each vacuole.[1] The nematocysts when they lie alone within the vacuoles are pointing indifferently in all directions, but after they have been taken charge of by the cnidophages they have in all instances their discharging poles directed towards the exterior of the body. Martin also observed this final exact orientation of the nematocysts. He says of it "the large barbed nematocysts in their final position, always lie so that the thread, when it is discharged, will pass out of the animal, although they may lie pointing in any direction while they are still in the gut cells or the body-cavity. This rule does not seem to hold good in the small cylindrical nematocysts, which, as far as I can see usually lie almost parallel to the surface. It is very difficult to say how such an orientation can be effected, but something of the same kind has been detected in *æolids*, and I believe that the same difficulty is present in the nematocysts of the tentacles in cœlenterates" (Martin, 1908, p. 267). It would indeed be a most difficult matter to understand their orientation if Hallez and Martin are correct in describing these exotic nematocysts as always lying within vacuoles. The cnidophages appear to be the vital agents which properly orient the nematocysts.

In my preparation one barbed nematocyst was observed that had been apparently inaccurately discharged. This was taken from near the middle of a specimen that when fixed remained extended. In such distended specimens the mesoderm near the middle of the animal is reduced to a very thin layer; this together with a possible shifting of the mesoderm at the exact time of discharge may reasonably account for this single exception that is represented in Fig. 11.

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[1] Hereafter we shall speak of this invading cell of the vacuole as a "*cnidophage*."

There is a second type of nematocyst found in *Hydra*. These are the so-called "grappling" nematocysts. By means of them the *Hydra* lays hold of or entwines objects of prey; when the prey is thus seized the tentacles of *Hydra* sweep it into the mouth of the polyp. This seems to be the chief function of these nematocysts; but the poison sac at the base of this type of nematocysts suggests that they are something more than mere prehensile structures. If they are not more than this, I wonder at *Microstoma* taking them into its tissues and passing them out to its surface where they are, so far as my observations are concerned, always oriented as are the barbed nematocysts. Frequent nematocysts of this type have been found by me within sections of *Microstoma*. In some cases these grappling nematocysts lie within vacuoles in which no attending cell or cnidophage can be recognized. Fig. 12 shows such a nematocyst lying near a vacuole from which it may have been discharged. These nematocysts are small and their vacuoles likewise small so that it is difficult to determine whether cells, associated with them, lie within their vacuoles or not; but frequent nematocysts of this type have been found that have attending cnidophages within their vacuoles (Fig. 13). Moreover all the exotic "grappling" nematocysts were so oriented that their discharging poles were directed towards the surface.

I have come to look upon the nematocysts of *Microstoma* as being derived from *Hydra*. This conclusion is based upon the following facts: (1) that there is a great variation in the number of nematocysts of *Microstome*—from very few to many; (2) the close resemblance between the nematocysts of *Hydra* and *Microstoma*; (3) the absence of cnidocils in *Microstoma;* and (4) the absence of cnidoblasts about the nematocysts when found within the endoderm and deeper mesoderm of *Microstoma*.

Toward what end is all this careful handling of the exotic nematocysts of *Microstoma* directed?

Glaser (1909) has determined by experiment that nematocysts will not be discharged in the digestive fluids of æolids.

There would be no protection gained by the flatworm in passing them into the tissue of its body if in the digestive fluids of *Microstoma* the nematocysts are likewise not discharged; and in case they did discharge within the digestive fluids of *Microstoma* they would have done their injury before they had been taken up by the tissues of the *Rhabdocœle*.

Glaser (1909) has further demonstrated that when nematocysts pass from digestive fluids into fresh water in all cases they discharge. Here then are seen grounds for making the inference that his careful handling of the nematocysts is done in order to prevent their haphazard discharge in the region of the mouth as they are egested. Over against this inference is the fact that in æolids certain nematocysts pass from the alimentary canal with the waste food while others are more carefully handled and discharged by the cnidophages of the cerata (Glaser, 1909): also when a flatworm is bold enough to attack a living *Hydra* with its anterior end it can hardly be considered to be so serious a matter to have nematocysts discharged casually within the vicinity of the mouth.

Again the end may be to avoid discharge of nematocysts by means of distortion within the enteron; for Glaser (1909) has shown that nematocysts when distorted do discharge. This suggestion I think can be dismissed with the inference that within the mesoderm the nematocysts are just as liable to distortion as within the enteron; for through the muscular actions of the body the ova are seen at times to suffer considerable distortion.

The third suggestion is that these bodies are taken up to be used. Grosvenor says: " 'No one can have witnessed the reaction of an æolid to various stimuli . . . without being convinced that the cerata are used as a means of defence' " (Glaser, 1910, p. 138). Likewise no one can have witnessed the discharge of nematocysts of *Microstoma* when stimulated by pressure or by acetic acid without looking upon them as organs of defense.

It taxes my faith seriously to believe that endodermal cells select nematocysts for so remote a purpose as that of defending the cell-colony at the ectodermal surface, and yet until further evidence can be obtained, if I must take a stand, I conclude that these exotic nematocysts are handled by *Microstoma* for purposes of defense.

If this conclusion is correct and if the nematocysts of æolids are derived from cœlenterata, the question can be raised, among many other more subtle ones, have the æolids acquired their method of dealing with nematocysts of cœlenterata through flatworm ancestry, or are these cases of parallel development of reflexes or instincts?

I hope to be able to make a series of experiments upon the nematocysts of these *Rhabdocœles*.

SUMMARY.

The nematocysts of *Microstoma* are derived from *Hydra* upon which *Microstoma* feeds. These ingested nematocysts are delivered to the mesoderm by the endoderm of the flatworm. Within the mesoderm an amœboid cell takes charge of each nematocyst and as it is transported to the surface orients it so that the "sting-thread" has its discharging pole directed towards the exterior of the animal. Thus *Microstoma* has come to use the nematocysts of its prey as do the æolids use the nematocysts of their prey.

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Explanation of Plate I.

Fig. 1. Nematocyst with its associated cell. Obtained by gentle pressure of cover-glass upon a specimen treated with a drop of 1 per cent. acetic acid. Free hand drawing, with aid of micrometer eye-piece. ×1,800.

Fig. 2. Same cell after lying ten minutes in the dilute acetic acid. Vesicle has formed at one end of cell. Free-hand drawing, with aid of micrometer eye-piece. ×1,800.

Fig. 3. Anterior end of *Chætogaster*. Free-hand drawing.

Fig. 4. Setæ taken from lumen of enteron of sectioned *Microstoma*. Camera lucida drawing. ×500.

Fig. 5. Endodermal cell. Nematocyst lying within vacuole, *V*, at base of cell; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

Fig. 6. Nematocyst within vacuole, *V*, lying near middle region of mesoderm; *A*, supporting cell of mesoderm; *B*, wandering cells of mesoderm; *E*, ectoderm; *M*, basement membrane of endoderm; *P*, pseudopodium (?) of wandering cell. Camera lucida drawing. ×1,500.

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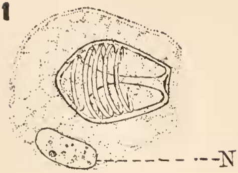

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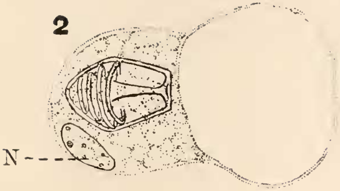

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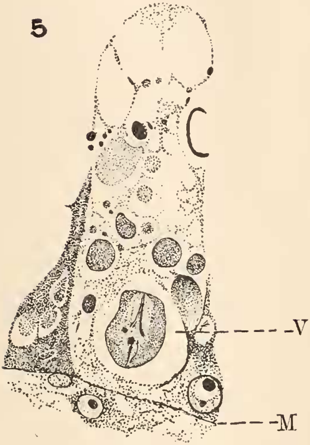

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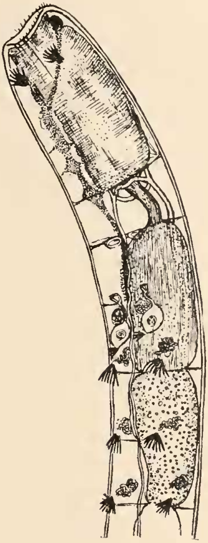

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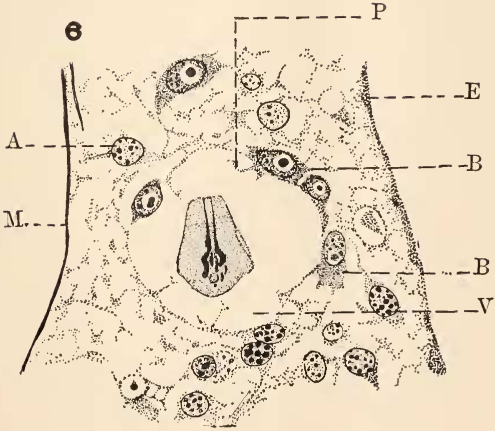

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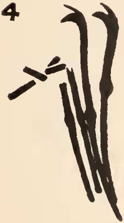

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